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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/82, C07K 14/415, C12N 5/10,</b> <b>A01H 5/00</b>	<b>A1</b>	<b>(11) International Publication Number: WO 99/15681</b>  <b>(43) International Publication Date: 1 April 1999 (01.04.99)</b>
<b>(21) International Application Number:</b> PCT/GB98/02850 <b>(22) International Filing Date:</b> 21 September 1998 (21.09.98)  <b>(30) Priority Data:</b> 9720039.8 19 September 1997 (19.09.97) GB  <b>(71) Applicant (for all designated States except US):</b> BIOGEMMA UK LIMITED [GB/GB]; Cambridge Science Park, Milton Road, Cambridge CB4 4GZ (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> PAUL, Wyatt [GB/GB]; Nickerson Biogemma Limited, Cambridge Science Park, Milton Road, Cambridge CB4 4GZ (GB). ROBERTS, Jeremy, Alan [GB/GB]; University of Nottingham, Division of Plant Science, School of Biological Sciences, Sutton Bon- ington Campus, Loughborough, Leicestershire LE12 5RD (GB). WHITELOW, Catherine [GB/GB]; University of Not- tingham, Division of Plant Science, School of Biological Sciences, Sutton Bonington Campus, Loughborough, Le- icestershire LE12 5RD (GB).  <b>(74) Agents:</b> CORNISH, Kristina, Victoria, Joy et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title: CONTROL POP DEHISCENCE OR SHATTER</b>		
<b>(57) Abstract</b>		
<p>This invention relates to the control of pod dehiscence or shatter. According to a first aspect of the present invention, there is provided nucleic acid, preferably recombinant or isolated nucleic acid sequence comprising the sequence as shown in figure 2 or a sequence substantially homologous thereto, or a fragment of the sequence as shown in figure 2. The fragment may comprise, in particular, the coding sequence as indicated in figure 2.</p>		

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## CONTROL POD DEHISCENCE OR SHATTER

This invention relates to the control of pod dehiscence or shatter.

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In the process of pod dehiscence, or shatter as it is commonly termed, degradation and separation of cell walls occurs along a discrete layer of cells, termed the dehiscence zone, and a localised increase in the activity of cellulase has been reported prior to the onset of dehiscence (Meakin and Roberts *J. Exp. Bot.* 41(229) 995-1002 (1990) and *J. Exp. Bot.* 41(229) 1003-1011 (1990)). This process is agronomically important because it may result in the premature shedding of seed before the crop can be harvested. Adverse weather conditions can exacerbate the process resulting in a greater than 50% loss of seed. This loss of seed not only has a dramatic effect on yield but also results in the emergence of the crop as a weed in the subsequent growing season.

15

Attempts to solve this problem over the last 20 years have focused on the breeding of shatter-resistant varieties. The most commonly used method is by trying to introduce germplasm from related species by interspecific hybridisation. Related species such as *B. nigra*, *B. juncea* and *B. campestris* have been used for this purpose but resulting plants from these crosses are frequently sterile and lose favourable characteristics which have to be regained by back crossing. This is both time consuming and laborious. The interspecific hybridisation strategy also has to cope with transferring two or more genes which are recessive in action into each of the breeding lines. Indeed, even within *B. campestris*, different genetic backgrounds have revealed different numbers of genes to be important in shatter resistance. This has necessitated breeders performing test crosses at each generation during the attempt to produce elite material. These difficulties have been compounded by the fact that shattering is a difficult and time-consuming trait to assess in the field. All these factors may account for the fact that the conventional breeding approach has made no progress over the last twenty years.

25  
30

Other methods employed to try and alleviate the problem include chemicals, in the

form of desiccants and pod sealants. The most widely used method to try and prevent seed loss is the mechanical technique of swathing in order to get uniform desiccation of the crop and reduce shattering by wind which occurs in the upright crop.

5

The present invention provides, by the use of recombinant technology, a further advantageous means for the control of pod dehiscence.

According to a first aspect of the present invention, there is provided nucleic acid, preferably recombinant or isolated nucleic acid sequence comprising the sequence as shown in figure 2 or a sequence substantially homologous thereto, or a fragment of the sequence as shown in figure 2. The fragment may comprise, in particular, the coding sequence as indicated in Figure 2. The nucleic acid is and/or the polypeptide which it encodes is involved in the process of pod dehiscence.

15

The invention has application to all crops that lose seed pre-harvest because of cell separation events. An economically important crop to which the invention applies is *Brassica napus*.

Recombinant or isolated nucleic acid sequences which are within the scope of the invention include those which, by virtue of the degeneracy of the genetic code, also code for the amino acid sequence indicated in figure 2. Nucleic acid sequences which are substantially homologous to nucleic acid sequences encoding the amino acid sequence shown in figure 2 also constitute preferred embodiments of the invention. "Substantial homology" may be assessed either at the nucleic acid level or at the amino acid level.

Any substantially homologous sequence should be at least 75%, preferably through 80%, 85%, 87, 90%, 95% identical to the coding sequence in Figure 2 at the nucleic acid residue level, using the default parameters of the BLAST 2.0 program of the National Centre for Biotechnology Information (available at <http://www.ncbi.nlm.nih.gov> or Altschul, S. F., *et al.*, Nucleic Acids Research, September 1997, 25(17), 3389-3402). Also covered by the present invention are

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sequences which comprise regions of complementary sequences to any sequence described above (that is any complementary sequence to the sequence shown in Figure 2, or a substantial homologue of such a sequence (as defined above)). Any fragment of the sequence (or of a sequence substantially homologous thereto, or of a complementary sequence thereto, including homologues) is at least 20 bases, preferably at least 30, 40, 50 or 60 bases in length.

At the nucleic acid level, sequences having substantial homology may also be regarded as those which are complementary to any of the previously described sequences (ie a sequence comprising a sequence as shown in Figure 2, or a substantial homologue thereof, or a fragment thereof). They may also be described as those sequences which hybridise to the nucleic acid sequences shown in Figure 2 under stringent conditions (for example at 35 to 65°C in a salt solution of about 0.9M) or conditions described in Plant Genetic Transformation and Gene Expression: A Laboratory Manual, Ed. Draper, J., *et al.*, 1988, Blackwell Scientific Publications, pp 252-255, modified as follows: prehybridization, hybridization and washes at 55 to 65°C, final washes (with 0.5X SSC, 0.1% SDS) omitted.

All references in this text to sequences includes within its scope substantially homologous sequences.

Fragments of the isolated or recombinant nucleic acid according to the invention are those which are functional and/or those which can be used in the regulation of plant dehiscence and/or those which can be used as tools, such as probes to identify homologous sequences (including orthologous sequences). Preferably, fragments are at least 20 bases, preferably at least 30, 40, 50, 60, 70, 80, or 90 bases in length. The invention most preferably relates to the full-length dehiscence zone protein a part of which is herein designated DZ15 as well as a nucleic acid sequence coding therefore, optionally including the promoter sequence.

Preferably, the nucleic acid of the invention will include a promoter or other regulatory sequence which naturally controls its expression. Since the sequence is expressed during dehiscence, such promoters are themselves useful in controlling

this process. Thus, in a further aspect the present invention provides a nucleic acid sequence which is a promoter or other regulatory sequence which naturally controls expression of the sequence herein described.

- 5 While promoters as described above may drive DNA encoding an enzyme, they may alternatively drive DNA whose transcription product is itself deleterious. Examples of such transcription products include antisense RNA and ribozymes.

As far as antisense nucleic acid is concerned, introducing the coding region of a  
10 gene in the reverse orientation to that found in nature can result in the down-regulation of the gene and hence the production of less or none of the gene product. The RNA transcribed from antisense DNA is capable of binding to, and destroying the function of, a sense RNA of the sequence normally found in the cell, thereby disrupting function. Examples of such antisense DNAs are the  
15 antisense DNAs of the sequence shown in Figure 2 and the sequence as claimed in claim 1. Since this gene is normally expressed in the dehiscence zone, antisense to it may be expected to disrupt normal dehiscence. Downregulation of the protein according to the present invention can be achieved by downregulation of the gene by partial or full (transwitch) sense expression.

20 Ribozymes are RNA "enzymes" capable of highly specific cleavage against a given target sequence (Haseloff and Gerlach, *Nature* 334 585-591 (1988)). The present invention also relates to ribozymes which are involved in or disrupt the correct transcription and expression of the dehiscence protein of the present invention.

25 Promoters useful as described above may be located in genomic libraries using, for example, probe sequences taken from the nucleic acid sequence of Figure 2 or as defined in claim 1 (by standard procedures as described below).

30 In another aspect, nucleic acid useful in the invention includes that which, when introduced into a plant, prevents or otherwise interferes with normal dehiscence by interfering with the normal plant expression. Of course, dehiscence-specific promoters, as discussed above, may be useful in this aspect of the invention.

However, there is a broader dimension which must be considered: antisense DNA or ribozyme-encoding DNA need not be driven by dehiscence-specific promoters. Instead, they could be driven by constitutive or other promoters (such as for example the nos, rubisco or plastocyanin promoter). If the sense gene is only expressed in the pod, there will, with an antisense approach, be no pleiotropic effects on plant development, and only the development of the dehiscence zone will be disrupted.

Antisense technology and ribozyme technologies have already found application in other areas of plant molecular biology. For example, antisense technology has been used to control tomato fruit ripening. Ribozyme technology has been used to control viral infection of melons. While DNA or RNA in accordance with this feature of the invention generally interferes with the plant's proper expression, in preferred embodiments expression is substantially prevented.

Another important aspect of the present invention is those nucleic acids which hybridise under stringent conditions to the nucleic acid sequences as claimed in any one of claims 1 to 5. For example, nucleic acid fragments are useful for probing for similar genes involved in dehiscence. For example, an *Arabidopsis* or other gene library may be probed. Fragments of the nucleic acid sequence shown in Figure 2, of at least 10, 20, 30, 40 or 50 more nucleotides may be used. Many useful probes are from 15 to 20 nucleotides in length.

In preferred embodiments of DNA sequences of this invention, 3'-transcription regulation signals, including a polyadenylation signal, may be provided. Preferred 3'-transcription regulation signals may be derived from the cauliflower mosaic virus 35S gene. It should be recognised that other 3'-transcription regulation signals could also be used.

The nucleic acid of the invention, preferably recombinant DNA in accordance with the invention may be in the form of a vector. The vector may for example be a plasmid, cosmid or phage. Vectors will frequently include one or more selectable markers to enable selection of cells transfected (or transformed: the terms are used



interchangeably in this specification) with them and, preferably, to enable selection of cells harbouring vectors incorporating heterologous DNA. Appropriate start and stop signals will generally be present. Additionally, if the vector is intended for expression, sufficient regulatory sequences to drive expression will be present; however, DNA in accordance with the invention will generally be expressed in plant cells, and so microbial host expression would not be among the primary objectives of the invention, although it is not ruled out. Vectors not including regulatory sequences are useful as cloning vectors.

10 Cloning vectors can be introduced into *E. coli* or another suitable host which facilitate their manipulation. According to another aspect of the invention, there is therefore provided a host cell transfected or transformed with DNA as described above.

15 DNA in accordance with the invention can be prepared by any convenient method involving coupling together successive nucleotides, and/or ligating oligo- and/or poly-nucleotides, including *in vitro* processes, but recombinant DNA technology forms the method of choice.

20 In the embodiments of the invention relating to dehiscence, the invention has application to all crops that lose seed pre-harvest because of cell separation events. An economically important crop to which the invention applies is *Brassica napus*. The invention is also particularly relevant to plants that develop dry fruits, including Brassica, Synapis and other genera of the Brassicaceae, soybean and other Leguminous species, Cuphea and sesame.

In preferred embodiments of DNA sequences of this invention, 3'-transcription regulation signals, including a polyadenylation signal, may be provided. Preferred 3'-transcription regulation signals may be derived from the cauliflower mosaic virus 30 35S gene. It should be recognised that other 3'-transcription regulation signals could also be used. Derivation of the full length sequence of DZ15 as well as 3' and 5' sequences can be carried out by standard procedures well known in the art. As a general procedure, sequences 5' to the coding sequence can be identified using the

following strategy: 1) use the DZ15 sequence (or part thereof) to probe a genomic library to obtain the full length clone, 2) locate the first ATG of the full length clone, 3) [optional] compare with other known sequences (such as on databases) to confirm the position of the ATG, 4) design primers to PCR a fragment from the ATG to a region upstream, preferably 1kb or more. Sequences 3' can be identified by an analogous process.

Ultimately, DNA in accordance with the invention will where appropriate be introduced into plant cells, by any suitable means. According to a further aspect of the invention, there is provided a plant cell including DNA in accordance with the first aspect of the invention as described above.

Preferably, DNA is transformed into plant cells using a disarmed Ti-plasmid vector and carried by *Agrobacterium* by procedures known in the art, for example as described in EP-A-0116718 and EP-A-0270822. Alternatively, the foreign DNA could be introduced directly into plant cells using an electrical discharge apparatus. This method is preferred where *Agrobacterium* is ineffective, for example where the recipient plant is monocotyledonous. Any other method that provides for the stable incorporation of the DNA within the nuclear DNA of any plant cell of any species would also be suitable. This includes species of plant which are not currently capable of genetic transformation.

Preferably DNA in accordance with the invention also contains a second chimeric gene (a "marker" gene) that enables a transformed plant containing the foreign DNA to be easily distinguished from other plants that do not contain the foreign DNA. Examples of such a marker gene include antibiotic resistance (Herrera-Estrella *et al.*, *EMBO J.* 2(6) 987-95 (1983) and Herrera-Estrella *et al.*, *Nature* 303 209-13 (1983)), herbicide resistance (EP-A-0242246) and glucuronidase (GUS) expression (EP-A-0344029). Expression of the marker gene is preferably controlled by a second promoter which allows expression in cells other than the dehiscence zone, thus allowing selection of cells or tissue containing the marker at any stage of regeneration of the plant. The preferred second promoter is derived

from the gene which encodes the 35S subunit of Cauliflower Mosaic Virus (CaMV) coat protein. However any other suitable second promoter could be used.

5 A whole plant can be regenerated from a single transformed plant cell, and the invention therefore provides transgenic plants (or parts of them, such as propagating material) including DNA in accordance with the invention as described above. The regeneration can proceed by known methods.

10 A further aspect of the invention is constituted by the newly isolated protein/polypeptide which is preferentially or exclusively expressed in dehiscence zones. Such a protein is that whose amino acid sequence is encoded, at least in part, by the sequence as set out in claim 1. This aspect of the invention provides a recombinant or isolated protein or polypeptide comprising the amino acid sequence as set out in Figure 2, or a sequence substantially homologous thereto, or a fragment  
15 of the amino acid sequence set out in Figure 2. Any substantially homologous sequence should be at least above 60% identical, preferably through 70%, 75%, 80%, 85%, 87%, 90%, 95% identical or at least above 76% similar, preferably through 80%, 85%, 90%, 95% similar (all) at the amino acid residue level, using the default parameters of the BLAST 2.0 program of the National Centre for Biotechnology  
20 Information (available at <http://www.ncbi.nlm.nih.gov> and also at the reference cited above alongside the web-site details). Any fragment should be of 6, preferably up to 10, 15 or 20 amino acid residues in length, of such a sequence. The invention also relates to a protein sequence which comprises the amino acid sequence as set out in Figure 2 and which is substantially the complete protein.

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The polypeptide /protein sequences of the present invention are particularly useful in developing tools, such as antibodies having specificity for identical or substantially homologous proteins/polypeptide sequences (including orthologous sequences) for further isolation or for identification of relevant sequences.

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The present invention also provides a nucleic acid sequence which encodes the amino acid sequence set out in Figure 2, or a substantial homologue thereof. The nucleic acid sequence may be the sequence set out in Figure 2 or may differ, taking into

account the degenerate nucleic acid code.

In yet a further aspect the invention provides the use of a recombinant or isolated nucleic acid sequence as described according to the first aspect, in the control of  
5 pod dehiscence. The use, in particular, is for the transformation of a plant, or of a plant part (such as propagating material) or a cell, to produce a transformed plant, plant part or plant cell, optionally to product plant propagating material.

The invention also provides a method of regulating dehiscence, which method  
10 comprises transforming propagating material from a plant with a nucleic acid sequence according to the first aspect of the invention.

Many of the techniques referred to herein use, or can use, hybridization to identify homologous sequences, or to probe for complementary sequences. Standard  
15 hybridization techniques can be used. For example, promoter sequences of interest can be used to identify and isolate substantially homologous promoters from other plants. Typically, a sequence is used, possibly a fragment thereof from 15 to 45 base pairs to hybridize to sequences from a suitable library under stringent conditions. Suitable conditions may be those described in Plant Genetic Transformation and  
20 Gene Expression: A Laboratory Manual, Ed. Draper, J., *et al.*, 1988, Blackwell Scientific Publications, pp 252-255, modified as follows: prehybridization, hybridization and washes at 55 to 65°C, final washes (with 0.5X SSC, 0.1% SDS) omitted.

25

Preferred features and details of each aspect (independent claim type) of the invention are as for each other aspect *mutatis mutandis*.

The present invention is supported and described with reference to the figures, in  
30 which:

Figure 1 shows Northern analysis of the expression of DZ15 in *B. napus* pods. A DZ15 riboprobe was generated using T3 DNA polymerase and

used to probe RNA isolated from pod dehiscence zones (DZ) or from pod valves lacking DZ (NZ). The blot was reprobed with the control probe 25S rRNA, to ensure that all lanes contained RNA.

5        Figure 2 shows the DNA sequence and putative amino acid sequence of DZ15.

The invention will now be illustrated by the following Examples.

10        EXAMPLE 1 – Identification and cloning of DZ15

Plant Material

Seeds of *B. napus* cv Rafal were grown as described by Meakin and Roberts, (*J. Exp. Bot.* 41(229) 995-1002 (1990)) with the following modifications. Single  
15        seedlings were potted into 10 cm pots containing Levington M2 compost, and after vernalisation for 6 weeks, were re-potted into 21 cm pots. Infection by powdery mildew or aphids was controlled by the application of Safers fungicide and insecticide. At anthesis, tags were applied daily to record flower opening. This procedure facilitated accurate age determination of each pod. Pods were harvested  
20        at various days after anthesis (DAA). The dehiscence zone was excised from the non-zone material and seed using a scalpel blade using the method of Meakin and Roberts (*J. Exp. Bot.* 41: 1003-1011 (1990)) and immediately frozen in liquid N<sub>2</sub> prior to storage at -70°C.

25        RNA Isolation

All chemicals were molecular biology grade and bought from either Sigma Chemical Ltd (Dorset, UK), or ICN Biomedicals. Total RNA was extracted using the polysomal extraction method of Christoffersen and Laties, *Proc. Natl. Acad. Sci.* 79 4060-4063 (1982), with the following alterations. The plant material was  
30        ground to a powder in liquid N<sub>2</sub> and then in 10 volumes of extraction buffer (200 mM Tris-acetate [pH 8.2], 100 mM magnesium acetate, 20 mM potassium acetate, 20 mM EDTA, 5% w/v sucrose, after sterilisation 2-mercaptoethanol was added to 15 mM and cycloheximide added to a final concentration of 0.1 mg ml<sup>-1</sup>). The

supernatant was then layered over 8 ml 1M sucrose made with extraction buffer and centrifuged in a KONTRON™ (Switzerland) TFT 70.38 rotor at 45,000 rpm (150,000 g) for 2 hr at 2°C in a Kontron CENTRIKON™ T-1065 ultra-centrifuge. Pellets were then resuspended in 500 µl 0.1M sodium acetate, 0.1% SDS, pH 6.0 and phenol/chloroform (1:1 v/v) extracted and the total RNA precipitated. mRNA was isolated from the total population of nucleic acids extracted from the dehiscence zone and non-zone tissue at 30 to 50 DAA pods, using the Poly(A) quick mRNA purification kit (Stratagene, Cambridge UK), and was used to make 1st strand cDNA using reverse transcriptase.

10

#### Differential display

This was performed essentially as described by Liang and Pardee (1992) [Liang, P. and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257, 967-971] using RNA extracted from 50 DAA (days after anthesis) pod dehiscence zones and non-zones. First strand cDNA copies of the RNAs (50 DAA DZ (non-dehiscence zone)/NZ (dehiscence zone) were made using 50U M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase (50U/µL) (Stratagene) in a 20µL reaction containing 1x M-MLV buffer, 2.5mM dNTPs (Pharmacia), 1µg RNA, 30U RNase inhibitor (Promega) and 10µM oligo dT anchor primer 8 (5'-TTTTTTTTTTTTTGA-3'). The reaction conditions were as follows: 65°C for 5 minutes, 37°C for 90 minutes and 95°C for 5 minutes. Following first strand cDNA synthesis, 60µL dH<sub>2</sub>O were added and the samples were either used directly for PCR or stored at -20°C.

25

For PCR, 2µL cDNA were used as template in a 20µL reaction containing 1x PCR buffer, 1mM MgCl<sub>2</sub>, 2µM dNTPs, 10µM oligo dT anchor primer 8 (5'-TTTTTTTTTTTTTGA-3'), 2.5µM arbitrary primer C (5'-AGGTGACCGT-3'), 0.5µL 35S-dATP (> 1000 Ci/mmol) (Amersham) and 1U *Taq* DNA polymerase (5U/µL) (Gibco BRL). The thermocycling conditions were as follows: 40 cycles of 94°C for 30 seconds, 40°C for 2 minutes, 72°C for 30 seconds followed by 72°C for 5 minutes. The PCR products were fractionated on a 5% polyacrylamide/7M urea gel after addition of 5µL loading buffer (95% (v/v)

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formamide, 20mM EDTA, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue) to each sample. Following electrophoresis the gel was dried at 80°C under vacuum for 1 hour then exposed to X-ray film (BioMax-MR, Kodak) in a light tight cassette for 48 hours. The dried gel and autoradiogram  
5 were aligned so that bands that appeared in the DZ and not in NZ could be cut out and the DNA eluted according to Liang et al. (1995) [Liang, P., Bauer, D., Averboukh, L., Warthoe, P., Rohrwild, M., Muller, H., Strauss, M. and Pardee, A. B. (1995) Analysis of altered gene expression by differential display. *Methods in Enzymology* 254, 304-321.]. The eluted PCR products (4µL) were  
10 reamplified in a 40µL reaction containing 1x PCR buffer, 1mM MgCl<sub>2</sub>, 20µM dNTPs, 10µM oligo dT anchor primer 8 (5'-TTTTTTTTTTTGA-3'), 2.5µM arbitrary primer C (5'-AGGTGACCGT-3') and 2U *Taq* DNA polymerase (5U/µL) (Gibco BRL) using the following thermocycling conditions: 40 cycles of 94°C for 30 seconds, 40°C for 2 minutes, 72°C for 30 seconds followed by 72°C  
15 for 5 minutes. The resulting PCR product was cloned into the TA cloning vector (Invitrogen) and sequenced [Figure 1]. In order to prepare an antisense strand-specific riboprobe, the PCR product was subcloned into pBluescript (Stratagene).

#### Expression pattern and analysis of DZ15.

20 Northern analysis using an antisense strand-specific riboprobe to the DZ15 PCR product, showed that DZ15 hybridised to a transcript of size 2.8kb to 3.4kb which was expressed in the DZ of 30-50 DAA pods (Figure 1). Minimal expression was observed in the pod NZ (Figure 1). A full-length DZ15 cDNA can be obtained by further screening of a cDNA library or using 5' RACE.  
25 Similarly the DZ15 gene and promoter can be obtained by standard techniques by screening a genomic library; for example a genomic library from *B.napus* or from a relative such as *Arabidopsis thaliana* can be employed.

The sequenced DZ15 PCR product (Figure 2) exhibits no extensive homology  
30 with any sequences in DNA databases.

EXAMPLE 2Production of shatter-resistant *B.napus* plants by antisense downregulation of DZ15

5 Any promoter that is expressed in the pod DZ during the time interval that DZ15 is expressed would be useful in a strategy to downregulate expression of the DZ15 gene product. Such a promoter is the DZ15 promoter. The DZ15 promoter was therefore obtained from an *A.thaliana* genomic library and linked to the DZ15 PCR product in a manner such that the DZ15 PCR fragment was in  
10 the antisense orientation. The resultant pDZ15-antiDZ15 gene was cloned into the binary vector pSCV nos nptII (SCV nos nptII is a derivative of pSCV1 (Firek *et al.* (1993), Plant Molecular Biology 22,129-142) which contains a nos promoter driving a Kanamycin resistance gene, cloned between the EcoRV and EcoRI sites of pSCV1). This binary construct was transformed into *B.napus*  
15 (var. Westar) by agrobacterial transformation essentially as described in Moloney M *et al.*, (1989) Plant Cell Reports 8, 238-242. A proportion of the resulting *B.napus* plants were found not to express DZ15 and consequently be resistant to pod shatter.



CLAIMS

1. A recombinant or isolated nucleic acid sequence comprising the sequence as shown in figure 2, or a sequence substantially homologous thereto, or a  
5 fragment of the sequence as shown in figure 2.
2. A nucleic acid sequence as claimed in claim 1, which includes a promoter or other regulatory sequence which controls expression of the sequence.
- 10 3. A nucleic acid sequence which is a promoter or other regulatory sequence which naturally controls expression of the sequence as claimed in claim 2.
4. A recombinant or isolated nucleic acid sequence which is antisense to a nucleic acid sequence as defined in any one of claims 1 to 3.
- 15 5. A nucleic acid sequence as claimed in claim 4 which includes a promoter or other regulatory sequence which naturally controls expression of the sequence as claimed in any one of claims 1 to 3.
- 20 6. A nucleic acid sequence which hybridises, under stringent conditions to a nucleic acid sequence as claimed in any one of claims 1 to 5.
7. A nucleic acid sequence as claimed in any one of claims 1 to 6 which is in the form of a vector.
- 25 8. A nucleic acid sequence as defined in any one of claims 1 to 7 which also includes a second chimeric or marker gene.
9. A nucleic acid sequence as claimed in claim 8, wherein the second gene is  
30 under the control of a second promoter which allows expression at any stage of plant regeneration.

10. A nucleic acid sequence as claimed in claim 9, wherein the second promoter is the nos promoter.
11. A host cell transformed or transfected with nucleic acid as claimed in any one of claims 1 to 10.
12. A plant cell which includes the nucleic acid as claimed in any one of claims 1 to 10.
13. A whole part or part of a plant, comprising a cell as claimed in claim 12.
14. A recombinant or isolated polypeptide comprising the amino acid sequence set out in Figure 2, or a substantially homologous sequence thereof, or a fragment thereof.
15. A transgenic plant which includes nucleic acid as defined in any one of claims 1 to 10 or a polypeptide or other sequence as claimed in claim 14.
16. Propagating material derived from a plant as claimed in claim 15.
17. A method of regulating dehiscence, which comprises the step of transforming or transfecting propagating material from a plant with a nucleic acid sequence as defined in any one of claims 1 to 10.
18. A method as claimed in claim 13 for reducing or preventing dehiscence, wherein the nucleic acid sequence is as defined in claim 4 or claim 6.
19. The use of a recombinant or isolated nucleic acid sequence as claimed in any one of claims 1 to 10 in the control of dehiscence.
20. The use as claimed in claim 15 wherein the nucleic acid sequence is used to transform a plant or plant propagating material to produce a transformed plant or plant propagating material.

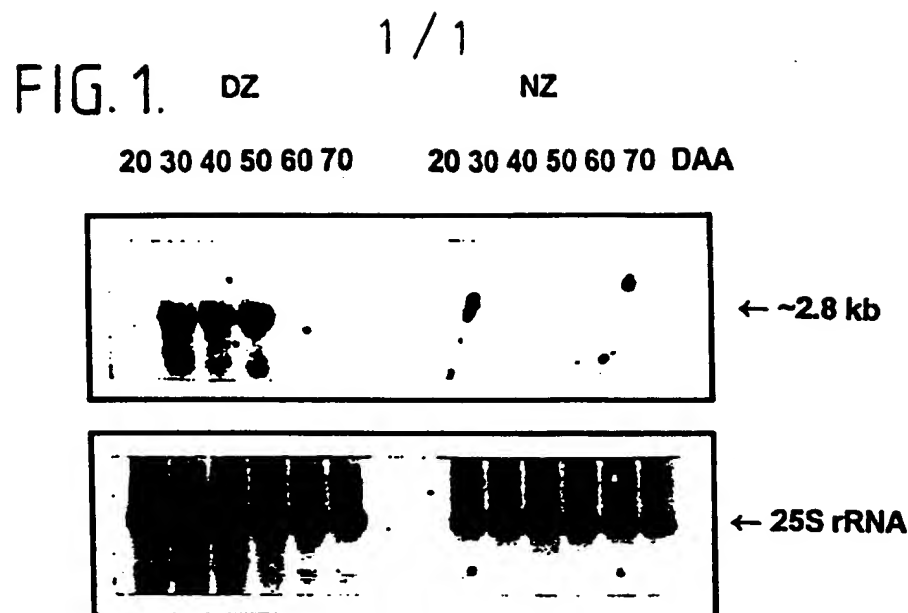


FIG. 2.

aggtgaccggttgctgatggcaatgtgctgggtcaagcgagaggttagacggtggcttggaga  
V T V A D G N V L V K R E V D G G L E T  
cagttaaagtcaaattgccagctgtcattagcgccgacttgctgggtcaatgagccgcggt  
V K V K L P A V I S A D L R L N E P R Y  
acgctactctgcccaatatcatgaaggccaagaagaagcccatcaaaaagctcacagcca  
A T L P N I M K A K K K P I K K L T A T  
cagatgtcgggtgtggacttggcgccacgtcaacaagtgttgagcgtagaagacccgcccc  
D V G V D L A P R Q Q V L S V E D P P T  
ccagacaggctgggtccattgtgcctgatgtcgacactctcatcaccaagttgaaagaaa  
R Q A G S I V P D V D T L I T K L K E K  
agggtcatttgtaatgcaatgtcaccaatacagttgttttagttcttataaaattcttcgt  
G H L \*  
gagggttttcagctgttaccaataatatTTTTTcaaaatcgattttattttacttgtaatt  
  
taaaagatcaaatattaatacaatgaacatttttgtaacagcaatcttttttttatattt  
  
tggagatttcatcgacttatgtcataattatTTTTtatcaatttattgttgttgttagtg  
  
atataataaagtatatTTTTctgggtcaaaa

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02850

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C07K14/415 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 23043 A (COUPE SIMON ALLAN ;ROBERTS JEREMY ALAN (GB); ISAAC PETER GEOFFREY) 13 October 1994 abstract; page 4,6,7; examples	1-15
A	WO 96 30529 A (NICKERSON BIOCEM LTD ;ROBERTS JEREMY ALAN (GB); COUPE SIMON ALLAN) 3 October 1996 see the whole document	1-15
A	WO 97 13865 A (PLANT GENETIC SYSTEMS NV ;ULVSKOV PETER (DK); CHILD ROBIN (GB); ON) 17 April 1997 see the whole document	1-15
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

21 January 1999

Date of mailing of the international search report

03/02/1999

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02850

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>JENKINS E S ET AL: "MOLECULAR CHARACTERISATION OF BRASSICA NAPUS POD DEHISCENCE"</p> <p>JOURNAL OF EXPERIMENTAL BOTANY,</p> <p>1 January 1995, page 42 XP000570274</p> <p>see the whole document</p> <p>-----</p>	1-15

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Information on patent family members

International Application No

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